The Chemical Composition of Cuticular Lipids from Dragonflies (Odonata)

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The cuticular lipids of 4 species *(Aeschna grandis, A. mixta, Sympetrum sanguineum, S. danae)* of the insect order Odonata (dragonflies) have been analysed. Alkanes, triglycerides, and free fatty acids predominate and minor amounts of monoester waxes have been detected. Among the hydrocarbons unbranched odd-numbered (56 – 66%) predominate, followed by monomethyl-alkanes with the branch in the middle of molecule (11 – 19%), 3-methylalkanes (7 – 15%), and 2-methyl-alkanes (1 – 13%). Moreover, alkanes (1 – 12%) were detected. The composition of triglycerides and free fatty acids were very similar with 14 : 0, 16 : 0, 18 : 0, 16 : 1, 18 : 1 and 18 : 2 being main constituents. Ester waxes were composed of unbranched and predominantly even-numbered fatty acids and alcohols with chain lengths $C_{14} - C_{30}$. The results are discussed from a chemotaxonomic viewpoint. Similarities of the integumental lipids from Odonata and Plecoptera were found.

Introduction

In the past, integumental lipids of various arthropods have been analysed, and it could be demonstrated that to a certain extent their patterns can be used as a chemotaxonomic parameter; *e. g.* within the order Coleoptera (beetles) hydrocarbons are the predominant constituents of the cuticular lipids the structure of which seems to be constant within one family, but differs from one family to another [1]. Hitherto only some of the 32 or 33 [2, 3] insect orders have been investigated in this regard including Plecoptera [4], Saltatoria [5], Blattodea [6], Hemiptera [7], Lepidoptera [8], Diptera [9], Hymenoptera [10], and Coleoptera [11]. Since no data were available on the order Odonata (dragonflies) and, moreover, their relationship to other insects is still under discussion, we decided to analyse the cuticular lipids of 4 species *(Aeschna grandis, A. mixta, Sympetrum sanguineum, S. danae)* belonging to two families (Aeschnidae and Libellulidae). The results allow a first comparison with two other orders close to Odonata (Plecoptera and Blattodea) in the natural system.

Materials and Methods

Material

The specimens (♀ and ♂ mixed) were collected in Skane/Sweden (*Aeschna grandis* (4 specimens), *A. mixta* (12 spec.), *Sympetrum danae* (33 spec.), and in Schleswig-Holstein (*Sympetrum sanguineum* (33 spec.)).

Methods

Animals were killed by deep-freezing and the cuticular lipids extracted by washing the specimens with chloroform for 5 seconds each. The extracts were filtered and separated into single lipid classes by column chromatography on SiO_2 (5 g, 9.8% water content). 70 ml cyclohexane eluted the aliphatic hydrocarbons, 100 ml cyclohexane/benzene (9 : 1; v/v) monoester waxes, 50 ml benzene/chloroform (9 : 1; v/v) triglycerides, and 50 ml chloroform free fatty acids.

Waxes, triglycerides, and free fatty acids were reesterified with 5% methanolic acid. In case of the waxes the resulting methyl esters and alcohols were separated by column chromatography on SiO_2. Alcohols were converted into fatty acids by CrO_3-oxidation [12] and subsequently esterified as above.

Gas-liquid chromatography was performed with a Perkin-Elmer F 20 FE instrument using 10 m glass columns with 3% OV 101 impregnation on Supelcoport at 250 °C (column, injection, and detection) and 2 m glass columns with 15% DEGS impregnation on GasChrom Q at 175 °C.

Mass spectra were recorded on a Varian-MAT 111 (GNOM) mass spectrometer at 80 eV using the

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Lipid	*Sympetrum sanguineum*	*Sympetrum danae*	*Aeschna grandis*	*Aeschna mixta*
	[% of total lipids]			
Hydrocarbons	29.2	26.3	2.6	5.4
Monoester waxes	5.2	3.3	5.6	4.5
Triglycerides	21.4	25.2	84.9	58.7
Free fatty acids	44.2	45.2	6.9	31.4

Table I. Quantitative composition of the lipids extracted from 4 *Odonata* species.

Hydrocarbon	*Sympetrum sanguineum*	*Sympetrum danae*	*Aeschna grandis*	*Aeschna mixta*
	% of the GLC peak area			
Unbranched (total)	(61.4)	(61.5)	(59.3)	(66.2)
n-C_{15}	–	–	–	0.3
n-C_{17}	–	–	–	0.1
n-C_{18}	–	–	–	0.1
n-C_{19}	–	–	–	trace
n-C_{20}	0.1	0.1	–	0.1
n-C_{21}	0.3	0.1	2.2	4.9
n-C_{22}	0.5	0.1	0.5	0.5
n-C_{23}	2.6	1.3	9.7	22.3
n-C_{24}	1.2	0.4	0.7	0.8
n-C_{25}	9.8	7.8	18.2	21.9
n-C_{26}	1.6	1.7	1.4	trace
n-C_{27}	24.4	28.3	19.8	11.7
n-C_{28}	0.5	2.0	trace	trace
n-C_{29}	20.4	17.0	6.8	3.5
n-C_{30}	–	trace	–	–
n-C_{31}	–	2.7	–	–
Alkenes (total)	(2.1)	(0.8)	(3.3)	(11.9)
$C_{23:1}$	0.7	0.3	1.1	4.2
$C_{25:1}$	1.4	0.5	2.2	5.6
$C_{27:1}$	–	–	–	2.1
2-Methylalkanes (total)	(5.1)	(11.5)	(2.7)	(0.5)
2-C_{24}	0.2	–	–	–
2-C_{25}	–	1.0	–	–
2-C_{26}	1.9	3.0	2.7	0.5
2-C_{27}	–	2.2	–	–
2-C_{28}	3.0	4.7	–	–
2-C_{30}	–	0.6	–	–
3-Methylalkanes (total)	(6.8)	(13.5)	(15.1)	(9.0)
3-C_{21}	–	–	–	0.1
3-C_{23}	0.2	–	0.7	1.3
3-C_{24}	–	0.5	–	1.3
3-C_{25}	1.5	2.0	2.7	3.7
3-C_{26}	–	–	3.1	–
3-C_{27}	4.9	8.0	8.6	2.6
3-C_{29}	0.2	3.0	–	–
Other Monomethylalkanes (total)	(18.8)	(11.3)	(18.8)	(12.0)
7-/9-C_{21}	–	–	–	0.1
7-/9-/11-C_{23}	–	–	–	0.5
5-C_{25}	–	–	1.3	1.6
9-/11-C_{25}	2.9	–	1.0	5.3
13-C_{25}	–	0.7	2.7	0.1
5-C_{27}	0.9	2.1	–	0.5
7-C_{27}	1.4	–	–	0.1
9-/11-/13-C_{27}	3.5	2.9	11.5	2.9
5-C_{29}	1.5	1.0	–	0.1
7-/9-C_{29}	1.5	–	–	0.1
11-/13-/15-C_{29}	7.1	3.2	2.3	0.7
11-/13-/15-C_{31}	–	1.4	–	–
unidentified	(5.8)	(1.4)	(0.8)	(0.4)

Table II. Quantitative composition of the cuticular hydrocarbons from 4 *Odonata* species.

Table III. Quantitative composition of the cuticular monoester wax constituents from 4 *Odonata* species.

Wax constituent	*Sympetrum sanguineum*	*Sympetrum danae*	*Aeschna grandis*	*Aeschna mixta*
	% of the GLC peak area			
Alkanols				
n-C_{14}	0.1	0.8	0.1	–
n-C_{15}	trace	0.1	0.1	–
n-C_{16}	2.9	3.1	2.3	1.3
n-C_{17}	0.5	0.4	0.2	0.9
n-C_{18}	4.9	4.1	2.2	2.4
n-C_{19}	0.1	0.7	0.6	0.5
n-C_{20}	6.3	5.8	3.8	4.2
n-C_{21}	0.1	0.2	1.5	0.3
n-C_{22}	10.2	9.5	6.1	9.2
n-C_{23}	0.1	0.1	0.8	1.2
n-C_{24}	14.2	12.9	26.0	37.6
n-C_{25}	0.1	0.1	0.6	0.6
n-C_{26}	3.8	4.7	12.2	14.4
n-C_{27}	0.1	0.1	0.5	0.5
n-C_{28}	16.7	10.7	19.1	8.7
n-C_{29}	0.1	0.1	0.8	0.8
n-C_{30}	35.7	39.2	22.5	15.6
unidentified	4.1	7.4	0.6	1.8
Fatty acids				
n-C_{12}	0.2	0.4	0.3	1.3
n-C_{14}	8.9	7.1	3.5	4.8
n-C_{15}	0.4	0.9	–	0.3
n-C_{16}	23.9	25.0	33.1	29.3
n-C_{17}	0.2	0.3	–	–
n-C_{18}	4.2	3.6	4.9	3.5
n-C_{20}	13.0	10.9	5.6	5.6
n-C_{21}	0.5	0.4	–	0.2
n-C_{22}	23.7	25.9	17.5	14.2
n-C_{23}	0.5	0.6	–	–
n-C_{24}	4.7	4.2	2.3	2.1
n-C_{26}	0.9	1.1	1.8	0.5
n-C_{28}	6.2	5.2	11.5	–
$C_{16:1}$	0.4	0.8	1.5	1.4
$C_{18:1}$	9.2	8.6	18.0	27.4
unidentified	3.1	5.0	–	9.4

Table IV. Quantitative composition of the cuticular triglycerides and free fatty acids from 4 *Odonata* species.

Fatty acid	*Sympetrum sanguineum*		*Sympetrum danae*		*Aeschna grandis*		*Aeschna mixta*	
	TG	FFA	TG	FFA	TG	FFA	TG	FFA
	% of the GLC peak area							
n-C_{10}	–	–	–	–	–	0.9	–	0.7
n-C_{12}	0.9	1.6	0.9	0.6	0.1	1.0	–	1.3
n-C_{14}	16.3	14.6	5.8	4.9	1.1	1.6	1.4	3.3
n-C_{15}	0.5	0.5	0.4	0.2	0.1	0.2	–	0.5
n-C_{16}	29.5	20.7	20.0	15.8	16.0	10.2	16.1	10.6
n-C_{18}	10.0	4.6	3.5	5.5	2.2	1.5	3.5	1.3
n-C_{20}	0.5	0.6	0.3	–	–	–	–	–
n-C_{21}	–	–	1.4	–	–	–	–	–
n-C_{23}	–	–	4.3	1.8	–	–	1.8	2.1
$C_{16:1}$	5.4	6.1	7.7	8.1	8.1	10.3	7.9	11.6
$C_{16:2}$	–	0.3	–	–	0.1	0.2	–	0.2
$C_{18:1}$	30.2	35.9	46.4	46.0	70.8	69.9	62.6	56.1
$C_{18:2}$	6.2	13.0	6.1	11.3	1.0	2.2	2.1	5.2
$C_{18:3}$	0.5	2.1	1.2	2.2	0.4	1.1	2.5	3.1
unidentified	–	–	2.0	3.6	0.1	0.9	2.1	4.0

TG, triglycerides; FFA, free fatty acids.

Ratio saturated/unsaturated	*Sympetrum sanguineum*	*Sympetrum danae*	*Aeschna grandis*	*Aeschna mixta*
Triglycerides				
$C_{16:0}/C_{16\ unsaturated}$	5.5	2.6	2.0	2.0
$C_{18:0}/C_{18\ unsaturated}$	0.2	0.6	0.03	0.05
Free fatty acids				
$C_{16:0}/C_{16\ unsaturated}$	3.4	1.9	0.99	0.91
$C_{18:0}/C_{18\ unsaturated}$	0.09	0.09	0.02	0.02

Table V. Degree of saturation of the cuticular triglycerides and free fatty acids from 4 *Odonata* species.

10 m OV 101 column. Details are published elsewhere [13].

Results and Discussion

Extraction and fractionation of the integumental lipids showed hydrocarbons, monoester waxes, triglycerides, and free fatty acids to be present in the 4 species investigated. The percentages (Table I) are very similar in both *Sympetrum* species, but differ significantly from *Aeschna.*

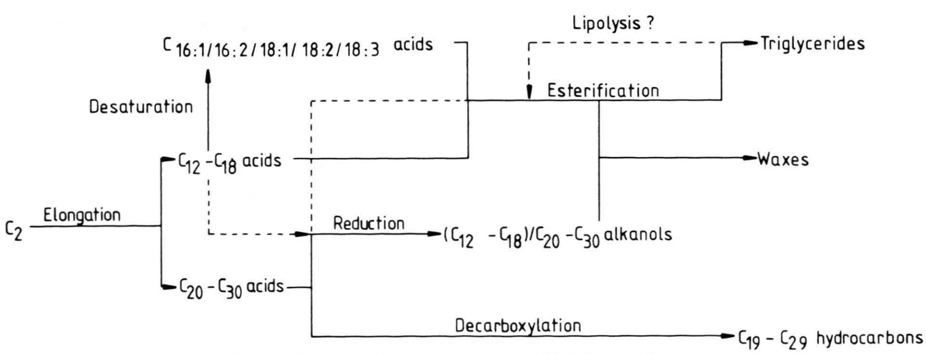
The qualitative composition of the single lipid classes is fairly uniform, although the two *Aeschna* species differ quantitatively from *Sympetrum* by possessing considerably higher amounts of oleic acid in the triglycerides and free fatty acids (Tables II – IV). Generally the degree of saturation is higher in *Sympetrum* than it is in *Aeschna.* Table V demonstrates, however, that the degree of saturation of the fatty acids is rather constant in species belonging to the same family.

Among the hydrocarbons odd-numbered individuals predominate in all homologeous series (*n*-alkanes, 3-, 5-, 7-, 9-, 11-, 13- and 15-methylalkanes, alkenes) except the 2-methylalkanes being mainly even-numbered. It is in accordance with earlier published data [14] that in this case isobutyric acid serves as initial substrate in the elongation of fatty acids which are subsequently converted into hydrocarbons by decarboxylation [15].

The ester wax constituents (alcohols and fatty acids) are predominantly even-numbered. It is remarkable that the alcohols show generally longer chains than the fatty acids; *e. g.* in both *Sympetrum* species the most abundant alcohol is triacontanol (C_{30}) and in the *Aeschna* species it is tetracosanol (C_{24}), whereas the corresponding acids are palmitic (C_{16}) and docosanoic acid (C_{22}). This may indicate that acids with medium chain lengths predominantly participate in the esterification process forming waxes, whereas acids with large chain lengths are alternatively converted into (a) alcohols by reduction, or into (b) hydrocarbons by decarboxylation. It, moreover, should be mentioned that no methyl-branched constituents could be detected in the waxes and triglycerides. They contribute to the hydrocarbon biosynthesis exclusively.

The main tentative biochemical pathways of the unbranched lipid constituents are summarized in the following scheme.

If the cuticular lipids of species from the order Odonata (dragonflies) are compared with those from other insect orders, only *Pteronarcys californica* (Ple-



Main tentative biochemical pathways of the unbranched lipid constituents.

coptera) shows a similar pattern [4] possessing hydrocarbons, ester waxes, triglycerides, and free fatty acids, although additional sterols have been detected in this species. The composition of the integumental lipids from all hitherto investigated Blattodea species differ more significantly [6, 17 – 19]. Actually, most of the arthropod systems locate Plecoptera next to Odonata and our findings agree very well with this. It has been stated, however, that there is no direct relationship between them [20]. It is questionable whether our findings have any systematic relevance or whether they reflect only the effect of adaptation to the more aquatic biotop of dragonflies. Armold *et al.* [4] *e. g.* have shown that the aquatic naiad and the terrestrial adult form of *Pteronarcys californica* differ significantly in their cuticular lipid pattern. On the other hand, we did not observe any characteristic difference in the integumental lipid composition of beetles living in moist or dry environments, respectively. Most of them possess exclusively hydrocarbons the structure of which, however, vary from one family to another. Integumental lipids of water beetles, however, have not been investigated systematically hitherto.

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